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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

* Chemical enhancement of virus transformation
* Herpes simplex type 2
* hydrazine
* 1,2-dimethydrazine
* norharman

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The temperature sensitive (ts) mutant $A_8$(293) of herpes simplex type 2 virus (HSV-2) was employed in tests to detect chemical interactions with the virus causing increased transformation of target cells. Mouse 3T3 cells were monitored for morphological transformation. Hydrazine and 1,2-dimethylhydrazine caused an enhancement of viral transformation of between three to four fold, confirming earlier findings using ultraviolet light inactivated wild type virus.

Norharman (pH-pyrido[3,4-6]indole), a component of tobacco tars, caused a nearly

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three-fold enhancement of transformation. Xylene and toluene were not found to enhance viral transformation. Experiments involving host-mediated activation of the test chemicals were unsuccessful because of low target cell recovery due to inhibition of the target 3T3 cells.

AFOSR-TR- 81-0633

ANNUAL TECHNICAL REPORT

TITLE: Effect of Chemical Mutagens on Herpes Virus - Induced Cellular Transformation and Testing for Mutagenesis in Mouse Cells

INVESTIGATOR: F. Brent Johnson, Ph.D.

INSTITUTION: Brigham Young University
Provo, Utah 84602

PROJECT PERIOD: 1 June 1980 to 31 May 1981

## ABSTRACT

The temperature sensitive (ts) mutant $A_8(293)$ of herpes simplex type 2 virus (HSV-2) was employed in tests to detect chemical interactions with the virus causing increased transformation of target cells. Mouse 3T3 cells were monitored for morphological transformation. Hydrazine and 1,2-dimethylhydrazine caused an enhancement of viral transformation of between three to four fold, confirming earlier findings using ultraviolet light inactivated wild type virus.

Norharman (9H-pyrido[3,4-6]indole), a component of tobacco tars, caused a nearly three-fold enhancement of transformation. Xylene and toluene were not found to enhance viral transformation. Experiments involving host-mediated activation of the test chemicals were unsuccessful because of low target cell recovery due to inhibition of the target 3T3 cells.

RESEARCH OBJECTIVES:

a. Determine if HSV-2-mediated transformation is enhanced following metabolic activation of the following chemicals:

dimethylnitrosamine (N-nitrosodimethylamine) (a known procaricinogen)
hydrazine
monomethylhydrazine
1,2-dimethylhydrazine
1,1-dimethylhydrazine
JP-5
JP-10
RJ-4
RJ-5

b. Determine if HSV-2-mediated transformation is enhanced when cells are simultaneously exposed to virus and the following suspected tumor promoters:

norharman
toluene
xylene

STATUS OF THE RESEARCH EFFORT: ACCOMPLISHMENTS.

A. Refinement of the Transformation Assay.

We previously reported the testing of a series of ts mutants of HSV-2 for transformation activity. The mutant ts Ag(293) was selected as an indicator strain to determine transformation enhancement. The method, as it is presently being used, is shown in Fig.1. This method avoids some of the pitfalls of the previous method, namely the sometimes erratic appearance of reactivated virus in UV-irradiated preparations.

B. Enhancement of Transformation by Hydrazine and 1,2-dimethylhydrazine Using the Mutant Virus Assay System.

We previously found enhancement of virus transformation by hydrazine (Hz) and symmetrical dimethylhydrazine (SDMH) in assays which employed UV-irradiated virus. The refined assay using the mutant virus had not been tested until this year when these tests were accomplished. (Refer to Table 1, Table 2, Fig. 2 and Fig. 3). These results show that virus interactions with Hz of SDMH (or Hz and SDMH catabolites) and the infected cell lead to greater numbers of transformed cells which grow out to recognizable foci.

C. Enhancement of Transformation by Norharman

Norharman, a compound found in tobacco tars and in tryptophan pyrolysates, was tested for enhancement. In other systems, alone, norharman has not mutagenic activity, but enhances the mutagenicity of several typical mutagens such as benzo [α] pyrene, dimethylaminoazobenzene and 2-acetyl-aminofluorene derivatives. Moreover, aniline and o-toluidine, which alone are non-mutagenic become mutagenic in the presence of norharman. The results of the present study show enhancement of herpes virus transformation in the presence of horharman when the chemical is added before the initiation of virus infection (Table 3, Fig. 4).

D. Lack of Enhancement by Xylene and Toluene.

Under the conditions and at the concentrations tested reagent grade xylene or toluene did not cause enhancement of virus transformation (Table 4, Table 5).

E. Host Mediated Activation of Chemicals.

The host mediated activation system of Hsie, et. al. (Mutation Res. 51: 77-84, 1978) was employed to determine whether prior activation of the chemicals would cause some of the previously negative chemicals to appear as positive virus enhancers. Balb/c mice were injected intraperitoneally with 3T3 cells and the chemical was injected subcutaneously. After two hours the cells were

removed from the peritoneal cavity, counted, plated and infected with virus. After four weeks, with weekly media changes, the cells were examined for the development of transformed foci. A major technical problem was encountered, i.e. the recovery of viable 3T3 cells from the peritoneal cavity. This was a problem even in control animals which received no chemical, so the loss of viability could not simply be ascribed to chemical toxicity. No matter how many cells were inoculated (up to $16 \times 10^6$) the recovery was always low. Even in the peritoneal cavity were extensively irrigated the recovery was low. Hence, the results were based upon formation of only limited numbers of clones. In these studies none of the previously negative chemicals showed up as a positive enhancer, but the nature of the technical difficulty prevents any conclusions being drawn, except that the animals caused loss of viability of the 3T3 cells, perhaps by allogeneic inhibition.

PUBLICATIONS:

1. Johnson, F. B. and J. R. Baker. 1981. Chemical enhancement of herpes simplex type 2-induced cellular transformation. Abstr. Ann. Meet. A.S.M., p. 226 (Abstract).

2. Johnson, F. B. 1981. Chemical interactions with herpes simplex type 2 virus: Enhancement of transformation by hydrazine and 1,2-dimethylhydrazine, (submitted).

PROFESSIONAL PERSONNEL ASSOCIATED WITH THE RESEARCH EFFORT:

1. F. Brent Johnson, Ph.D., principal investigator.

2. Joyce R. Baker, Ph.D., research technician.

INTERACTIONS:

Participation in and presentation of a report at the Review of Air Force Sponsored Basic Research in Environmental Toxicology, 2-3 June 1981, Columbus, Ohio.

NEW DISCOVERIES STEMMING FROM THE RESEARCH EFFORT:

1. Supportive evidence was obtained for the transformation enhancing activity of hydrazine and 1,2-dimethyl-hydrazine in assays involving the mutant virus.

2. That norharman enhances the transformation potential of herpes simplex type 2 virus.

3. That xylene and toluene were negative in enhancement assays.

## ENHANCEMENT ASSAY

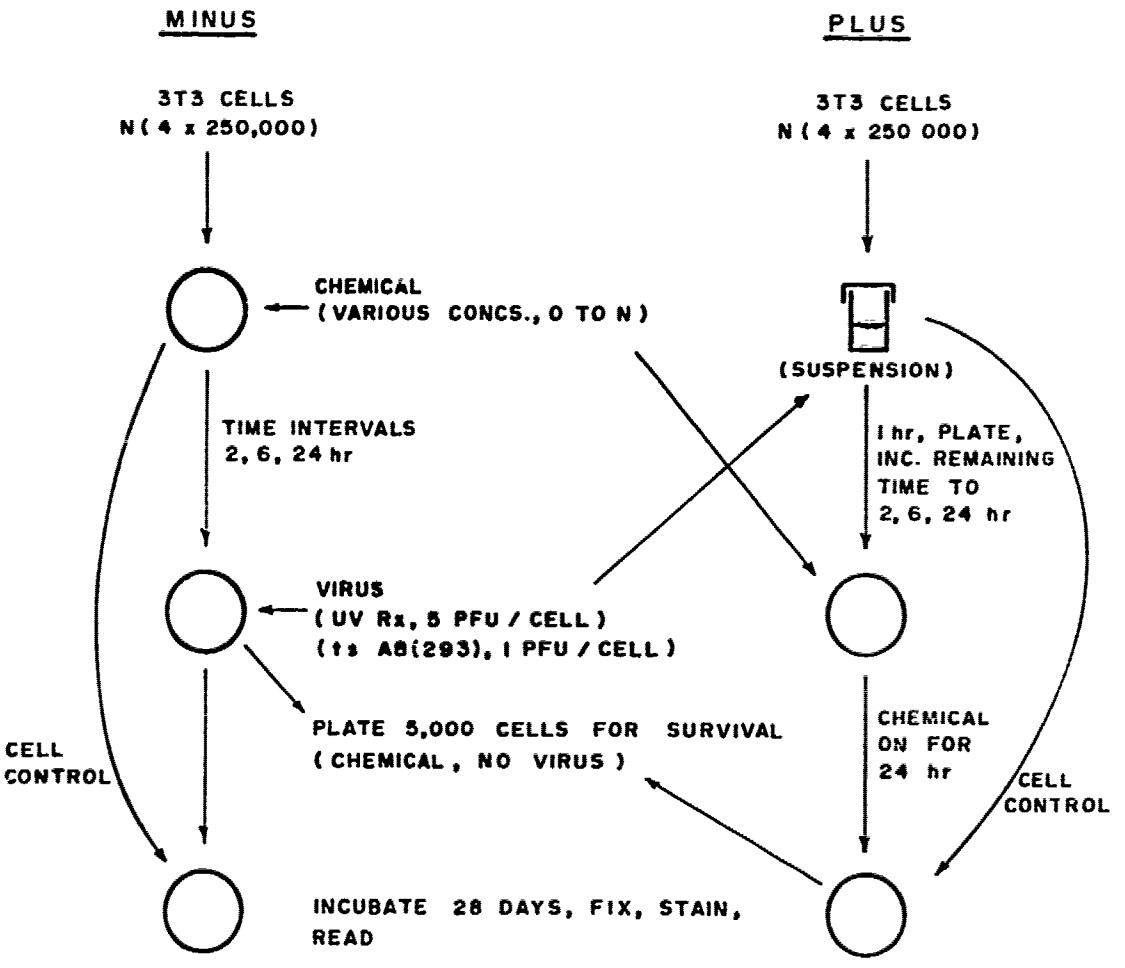


Fig. 1. Assay scheme for the determination of chemical enhancement of herpes virus transformation. The "minus" system refers to exposure of the cells to the chemical prior to virus infection. The "plus" system indicates exposure to the chemicals after virus infection. For each concentration of chemical to be tested (from 0 to N number of concentrations) four petri dishes are seeded with 250,000 cells in each dish. Some dishes are set aside as cell controls. The chemical, contained in cell culture medium, is added to the cells for the length of time to be tested. Some cells are removed and plated for survival to determine chemical toxicity. At the end of the chemical exposure time the media are removed, the cell sheets washed with PBS, the virus added and allowed to absorb for one hour then fresh media added to the cultures. In the "plus" system, the chemical is on for 24 hrs., then washed away and the cells renewed with fresh media. At the end of the incubation period the cell sheets are examined for foci of morphologically transformed cells.

TABLE 81. ENHANCEMENT OF HSV-2 ts $A_8$(293) TRANSFORMATION OF 3T3 CELLS BY SDMH

| Time of carcinogen addition (h) | SDMH (μg/ml) | Total colonies per 5000 cells[b] | Surviving fraction[c] | Transformed foci/$10^6$ cells | Transformation frequency[d] | Enhancement ratio[e] |
|---|---|---|---|---|---|---|
| -24[a] | 1.0 | 798 | 0.9710 | 41 | 42.22 | 3.84 |
| | 0.5 | 744 | 0.9050 | 13 | 14.36 | 1.31 |
| | 0.1 | 870 | 1.0584 | 2 | 1.89 | 0.17 |
| | 0.05 | 755 | 0.9180 | 11 | 11.98 | 1.09 |
| | 0 | 822 | 1.00 | 11 | 11.00 | 1.00 |
| -6 | 5.0 | 714 | 0.8470 | 0 | - | - |
| | 1.0 | 614 | 0.7284 | 0 | - | - |
| | 0.5 | 542 | 0.6429 | 0 | - | - |
| | 0.1 | 476 | 0.5447 | 0 | - | - |
| | 0.05 | 410 | 0.4864 | 0 | - | - |
| | 0 | 843 | 1.00 | 2 | 2.00 | 0.50 |
| -2 | 5.0 | 436 | 0.7415 | 2 | 2.70 | 0.68 |
| | 1.0 | 431 | 0.7330 | 2 | 2.73 | 0.68 |
| | 0.5 | 404 | 0.6871 | 2 | 2.91 | 0.73 |
| | 0.1 | 435 | 0.7398 | 4 | 5.41 | 1.35 |
| | 0.05 | 503 | 0.8554 | 0 | - | - |
| | 0 | 588 | 1.00 | 3 | 3.00 | 0.75 |
| +2 | 5.0 | 566 | 0.9826 | 0 | - | - |
| | 1.0 | 591 | 1.0260 | 0 | - | - |
| | 0.5 | 725 | 1.2587 | 2 | 1.59 | 0.40 |
| | 0.1 | 567 | 0.9844 | 0 | - | - |
| | 0.05 | 475 | 0.8247 | 1 | 1.21 | 0.30 |
| | 0 | 576 | 1.00 | 9 | 9.0 | 2.25 |
| +6 | 5.0 | 658 | 1.1544 | 0 | - | - |
| | 1.0 | 475 | 0.8333 | 2 | 2.40 | 0.60 |
| | 0.5 | 626 | 1.0982 | 1 | 0.91 | 0.23 |
| | 0.1 | 389 | 0.6825 | 3 | 4.40 | 1.10 |
| | 0.05 | 376 | 0.6596 | 0 | - | - |
| | 0 | 570 | 1.00 | 4 | 4.00 | 1.00 |
| +24 | 5.0 | 409 | 0.9761 | 1 | 1.02 | 0.26 |
| | 1.0 | 348 | 0.8305 | 2 | 2.41 | 0.60 |
| | 0.5 | 429 | 1.0239 | 2 | 1.95 | 0.49 |
| | 0.1 | 448 | 1.0692 | 3 | 2.81 | 0.70 |
| | 0.05 | 459 | 1.0955 | 0 | - | - |
| | 0 | 419 | 1.00 | 2 | 2.00 | 0.50 |

[a] A negative time refers to treatment of the cells with chemical before virus infection. Positive times refer to treatment of the cells after virus infection.

[b] Numbers of surviving cells after chemical treatment.

[c] Number of survivors divided by number of colonies in non-treated cultures.

[d] Number of transformed foci per $10^6$ cells divided by the surviving fraction.

[e] The transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

TABLE II: ENHANCEMENT OF HSV-2 ts $A_n$ (293) Transformation of 3T3 Cells by Hydrazine

| Time of carcinogen addition (h) | HZ (µt/ml) | Total colonies per 5000 cells[b] | Surviving fraction[c] | Transformed foci/$10^6$ cells | Transformation frequency[d] | Enhancement ratio[e] |
|---|---|---|---|---|---|---|
| -24[a] | 0.05 | 95 | 0.3276 | 4 | 12.21 | 3.46 |
| | 0.01 | 187 | 0.6448 | 7 | 10.86 | 3.07 |
| | 0.005 | 341 | 1.1800 | 5 | 4.24 | 1.20 |
| | 0.001 | 198 | 0.6828 | 2 | 2.93 | 0.83 |
| | 0 | 290 | 1.00 | 4 | 4.0 | 1.13 |
| -6 | 0.05 | 400 | 1.3600 | 1 | 2.94 | 0.83 |
| | 0.01 | 454 | 1.5400 | 3 | 3.90 | 1.10 |
| | 0.005 | 453 | 1.5400 | 2 | 0.97 | 0.27 |
| | 0.001 | 284 | 0.9659 | 5 | 5.18 | 1.47 |
| | 0 | 294 | 1.00 | 0 | - | - |
| -2 | 0.05 | 585 | 1.2300 | 4 | 3.25 | 0.92 |
| | 0.01 | 471 | 0.9937 | 1 | 1.01 | 0.29 |
| | 0.005 | 416 | 0.8776 | 2 | 2.28 | 0.65 |
| | 0.001 | 414 | 0.8734 | 2 | 2.29 | 0.65 |
| | 0 | 474 | 1.00 | 3 | 3.00 | 0.85 |
| +2 | 0.05 | 472 | 1.0600 | 4 | 3.77 | 1.06 |
| | 0.01 | 471 | 1.0600 | 7 | 6.60 | 1.87 |
| | 0.005 | 462 | 1.0400 | 4 | 3.85 | 1.09 |
| | 0.001 | 462 | 1.0400 | 0 | - | - |
| | 0 | 445 | 1.00 | 2 | 2.00 | 0.57 |
| +6 | 0.05 | 405 | 0.0299 | 0 | - | - |
| | 0.01 | 445 | 0.9119 | 6 | 6.58 | 1.86 |
| | 0.005 | 522 | 1.07 | 2 | 1.87 | 0.53 |
| | 0.001 | 450 | 0.9221 | 5 | 5.42 | 1.53 |
| | 0 | 488 | 1.00 | 4 | 4.00 | 1.13 |
| +24 | 0.05 | 405 | 0.6860 | 5 | 7.29 | 2.06 |
| | 0.01 | 538 | 0.7610 | 6 | 7.88 | 2.23 |
| | 0.005 | 555 | 0.7850 | 3 | 3.82 | 1.08 |
| | 0.001 | 525 | 0.7426 | 3 | 4.04 | 1.14 |
| | 0 | 707 | 1.00 | 5 | 5.00 | 1.41 |

[a] A negative time refers to treatment of the cells with chemical before virus infection. Positive times refer to treatment of the cells after virus infection.

[b] Numbers of surviving cells after chemical treatment.

[c] Number of survivors divided by number of colonies in non-treated cultures.

[d] Number of transformed foci per $10^6$ cells divided by the surviving fraction.

[e] The transformation frequency of the chemically treated cultures divided by the transformation frequency of the non-treated cultures.

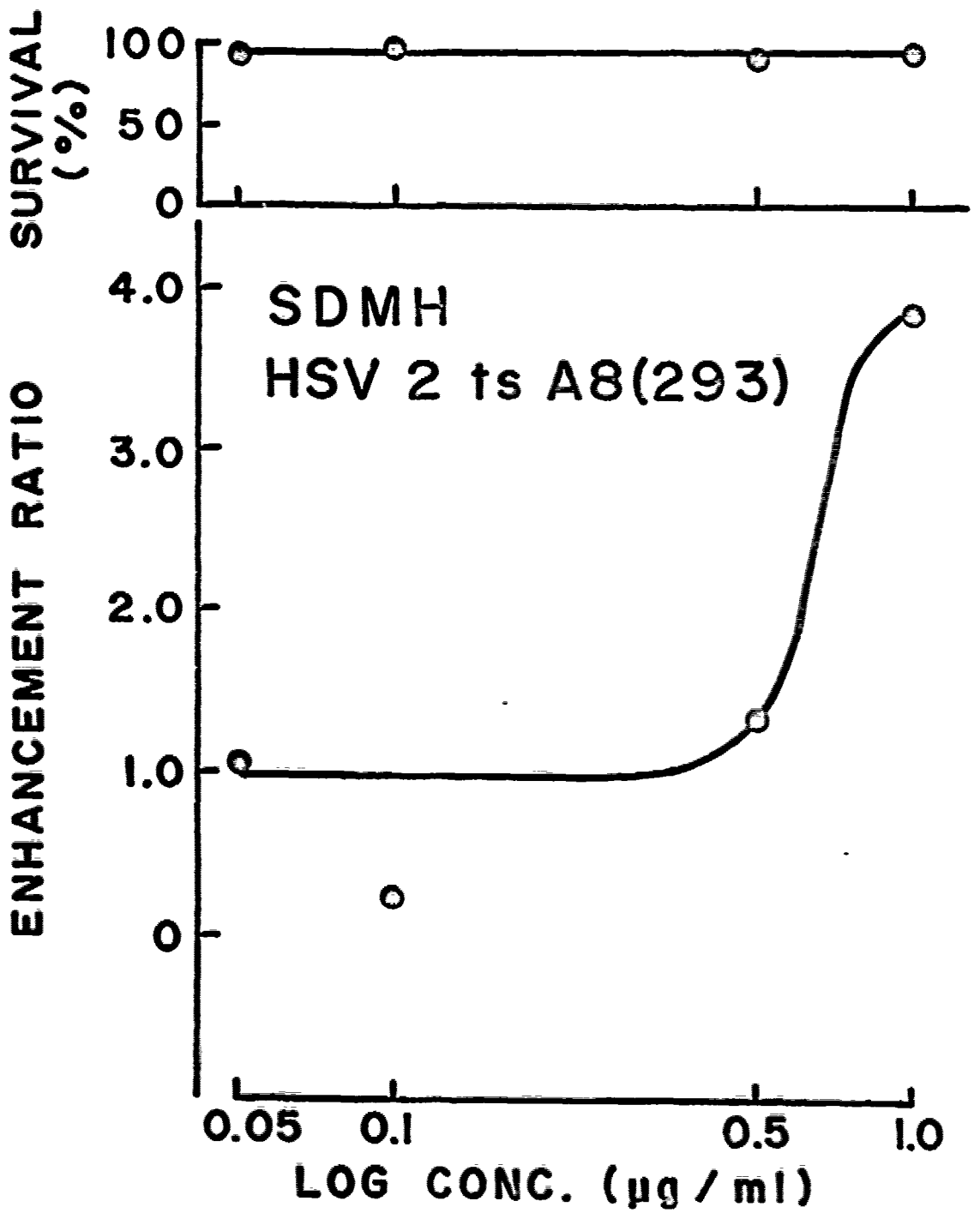
SURVIVAL (%)
100
50
0
ENHANCEMENT RATIO
4.0
3.0
2.0
1.0
0
SDMH
HSV 2 ts A8(293)
0.05
0.1
0.5
1.0
LOG CONC. (μg / ml)

Fig 2.

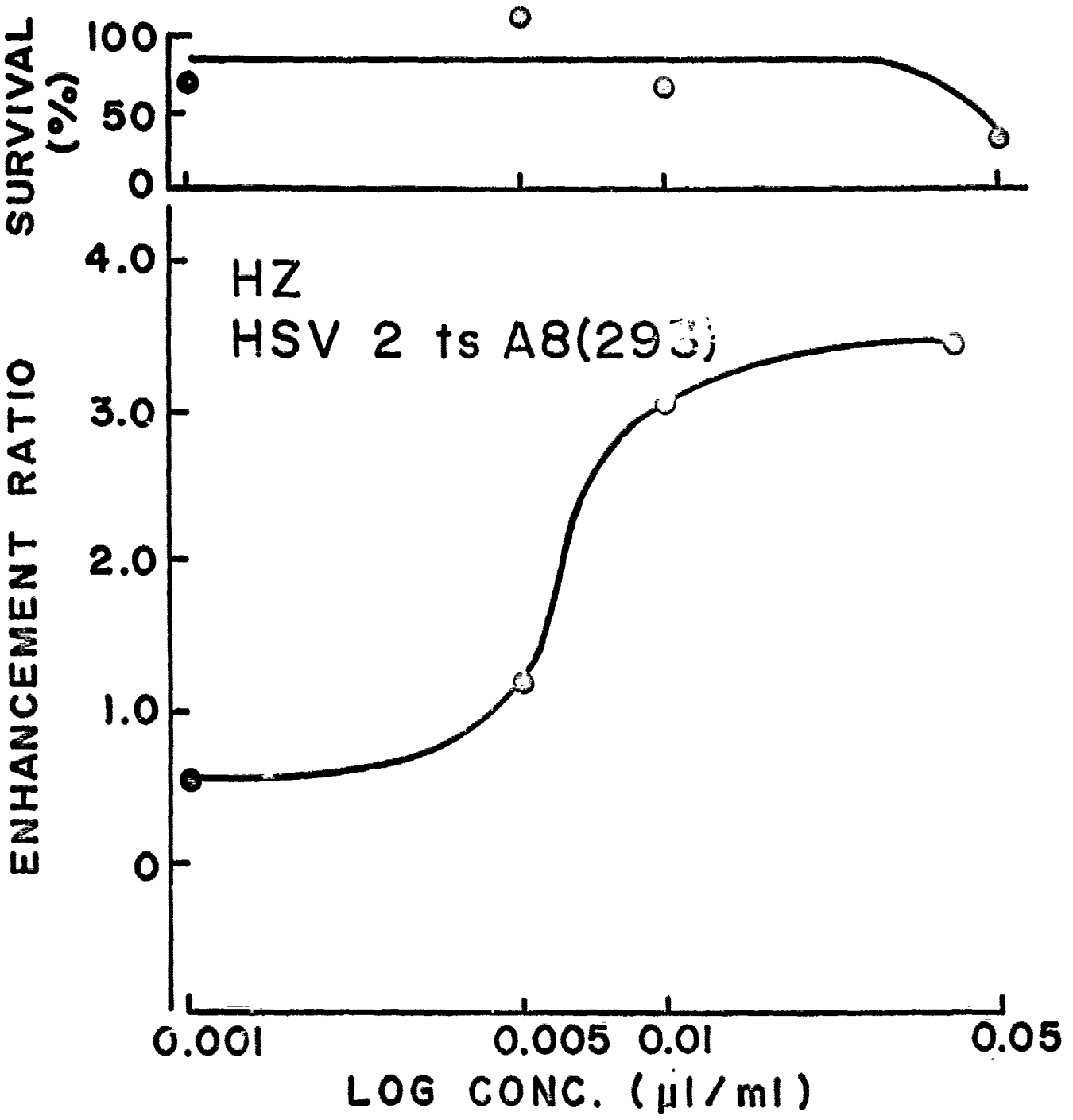
SURVIVAL (%)
100
50
0
ENHANCEMENT RATIO
4.0
3.0
2.0
1.0
0
HZ
HSV 2 ts A8(293)
0.001
0.005
0.01
0.05
LOG CONC. (µl/ml)

TABLE 3. Enhancement of HSV-2 Transformation by Norharman

| Time [a] (hr) | Conc. (μg/ml) | Survivors per 5000 cells | Surviving Fraction | Transformed Foci/$10^6$ cells | Transformation Frequency | Enhancement Ratio |
|---|---|---|---|---|---|---|
| -24 | 50 | 535 | 0.9817 | 34 | 34.63 | 0.84 |
| | 10 | 518 | 0.9505 | 92 | 96.79 | 2.36 [b] |
| | 5 | 488 | 0.8954 | 106 | 118.38 | 2.88 |
| | 1 | 481 | 0.8826 | 67 | 75.91 | 1.85 |
| | 0.5 | 477 | 0.8752 | 85 | 97.12 | 2.37 |
| | 0.1 | 517 | 0.9486 | 35 | 36.90 | 0.90 |
| | 0 | 545 | 1.00 | 41 | 41.00 | 1.00 |
| | N | 485 | | 0 | | |
| +24 | 50 | 344 | 0.6478 | 37 | 57.12 | 1.44 |
| | 10 | 365 | 0.6874 | 35 | 50.92 | 1.27 |
| | 5 | 333 | 0.6271 | 42 | 66.97 | 1.67 |
| | 1 | 417 | 0.7853 | 52 | 66.22 | 1.66 |
| | 0.5 | 476 | 0.8964 | 54 | 60.24 | 1.51 |
| | 0.1 | 501 | 0.9435 | 34 | 36.04 | 0.90 |
| | 0 | 531 | 1.00 | 40 | 40.00 | 1.00 |
| | N | 551 | | 0 | | |

[a] Relative time of the addition of the chemical

[b] ═══ (Double underline) = Significant at the 1% confidence level using Casto values

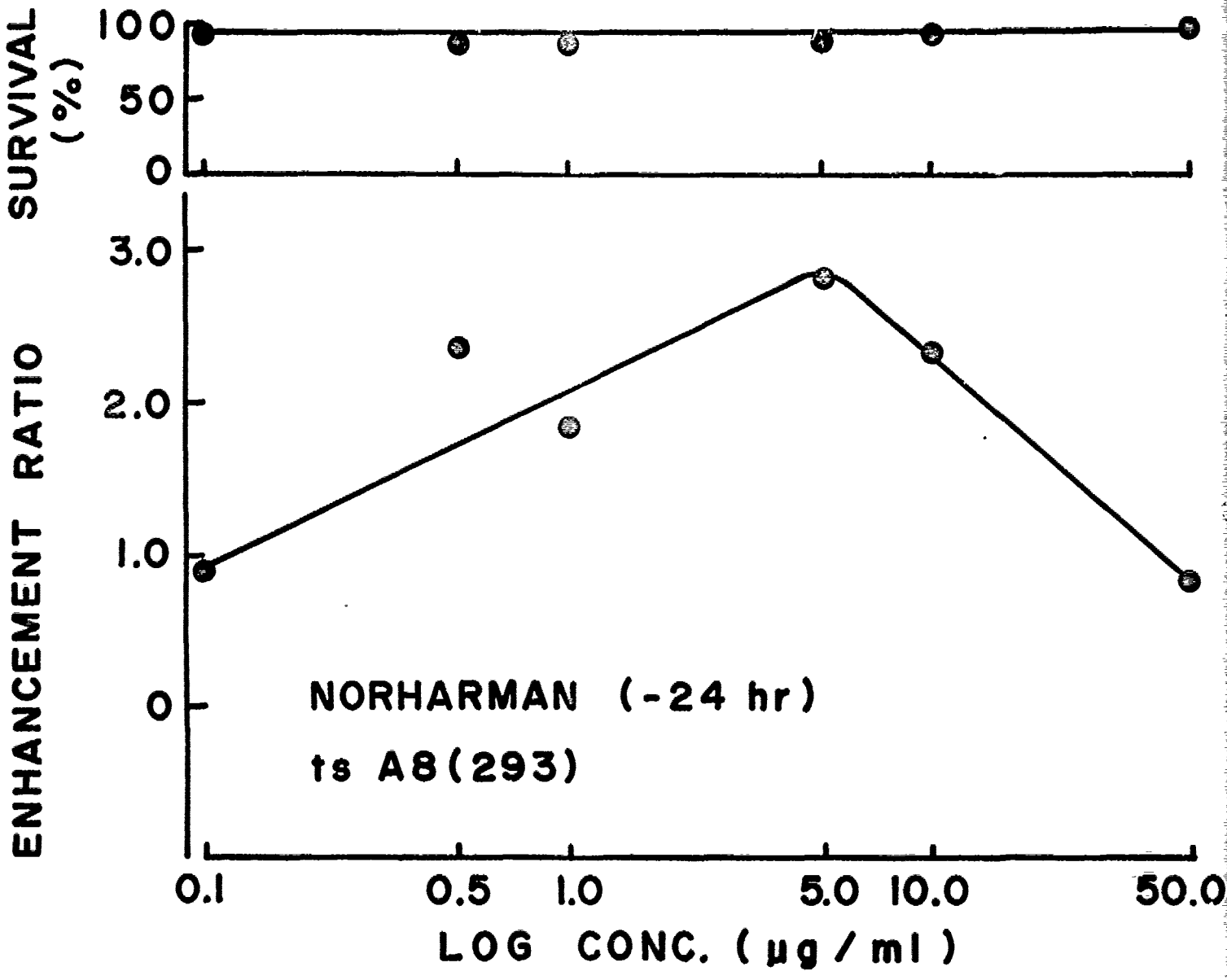
SURVIVAL (%)
100
50
0
ENHANCEMENT RATIO
3.0
2.0
1.0
0
NORHARMAN (-24 hr)
ts A8(293)
0.1
0.5
1.0
5.0
10.0
50.0
LOG CONC. (μg / ml)

Fig. 4

TABLE II. Lack of Enhancement of HSV-2 Transformation by Xylene

| Time [a] (hr) | Conc. (µl/ml) | Survivors per 5000 cells | Surviving Fraction | Transformed Foci | Transformation Frequency | Enhancement Ratio |
|---|---|---|---|---|---|---|
| -6 | 0.5 | 498 | 1.0873 | 29 | 26.7 | 1.03 |
| | 0.1 | 370 | 0.8079 | 20 | 24.76 | 0.95 |
| | 0.05 | 484 | 1.0568 | 23 | 21.76 | 0.84 |
| | 0.01 | 432 | 0.9432 | 24 | 25.45 | 0.98 |
| | 0.005 | 422 | 0.9214 | 24 | 26.05 | 1.00 |
| | 0 | 458 | 1.00 | 26 | 26.00 | 1.00 |
| | N [b] | 414 | | 0 | | |
| +6 | 0.5 | 259 | 1.0319 | 96 | 93.03 | 1.11 |
| | 0.1 | 229 | 0.9124 | 100 | 109.60 | 1.30 |
| | 0.05 | 243 | 0.9681 | 80 | 82.64 | 0.98 |
| | 0.01 | 210 | 0.8367 | 88 | 105.18 | 1.25 |
| | 0.005 | 141 | 0.5618 | -- | -- | -- |
| | 0 | 251 | 1.00 | 84 | 84.00 | 1.00 |
| | N [b] | 265 | | 0 | | |
| -24 | 0.5 | 681 | 0.9799 | 144 | 146.95 | 1.20 |
| | 0.1 | 612 | 0.8806 | 149 | 169.20 | 1.39 |
| | 0.05 | 669 | 0.9626 | 146 | 151.67 | 1.24 |
| | 0.01 | 573 | 0.8245 | 159 | 192.84 | 1.58 |
| | 0.005 | 681 | 0.9799 | 159 | 162.26 | 1.33 |
| | 0 | 695 | 1.00 | 122 | 122.00 | 1.00 |
| | N [b] | 736 | | 0 | | |
| +24 | 0.5 | 365 | 1.1661 | 95 | 81.47 | 0.94 |
| | 0.1 | 358 | 1.1438 | 90 | 78.69 | 0.90 |
| | 0.05 | 311 | 0.9936 | 79 | 79.51 | 0.91 |
| | 0.01 | 322 | 1.0288 | 81 | 78.73 | 0.90 |
| | 0.005 | 304 | 0.9712 | 77 | 79.28 | 0.91 |
| | 0 | 313 | 1.00 | 87 | 87.00 | 1.00 |
| | N [b] | 351 | | 0 | | |

[a] Time of addition of xylene

[b] N = Cell Control (cells exposed to neither chemical nor virus)

TABLE 5. Lack of Enhancement of HSV-2 Transformation by Toluene

| Time [a] (hr) | Conc. (µl/ml) | Survivors per 5000 cells | Surviving Fraction | Transformed Foci | Transformation Frequency | Enhancement Ratio |
|---|---|---|---|---|---|---|
| -6 | 0.5 | 788 | 0.9575 | 13 | 13.58 | 1.04 |
| | 0.1 | 866 | 1.0522 | 15 | 14.26 | 1.10 |
| | 0.05 | 772 | 0.9380 | 13 | 13.86 | 1.07 |
| | 0.01 | 926 | 1.1252 | 13 | 11.55 | 0.89 |
| | 0 | 823 | 1.00 | 13 | 13.00 | 1.00 |
| | N [b] | 712 | | 0 | | |
| -24 | 0.5 | 417 | 0.4528 | 7 | 15.46 | 0.97 |
| | 0.1 | 438 | 0.4756 | 6 | 12.62 | 0.79 |
| | 0.05 | 689 | 0.7481 | 8 | 10.69 | 0.67 |
| | 0.01 | 846 | 0.9186 | 9 | 9.80 | 0.61 |
| | 0 | 921 | 1.00 | 16 | 16.00 | 1.00 |
| | N [b] | 911 | | 0 | | |

[a] Time of addition of toluene

[b] N = Cell control (cells exposed to neither chemical, nor virus)